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Viability of 1+1 Douglas-fir Transplants When Held in Cold or Frozen Storage

ABSTRACT: Cold and frozen storage treatments were evaluated for 1+1 Douglas-fir transplants lifted in January 1994 at J. Herbert Stone Nursery. For one seedlot of transplants in average condition, five treatments included continuous cold, continuous frozen, or combinations of cold and frozen storage. Five additional seedlots, exhibiting worse than average chlorosis and needle and twig mortality, were compared under continuous cold or continuous frozen storage.

Seedlings were outplanted at J. Herbert Stone Nursery in May 1994, and evaluated for survival the following October.

For the seedlot of average quality, the five cold and frozen storage treatments did not result in differences in percentage of roots diseased, or of number of seedlings with spots or small patches of necrotic cambium (mottling), by May.

For the other five seedlots of below average quality, percentage of roots diseased tended to increase with time in cold storage while remaining constant in frozen storage. For all seedlots, the number of seedlings with mottling was significantly greater ($P < 0.01$) after three months of cold storage, compared with that after three months of frozen storage. With regard to mottling, seedlings in frozen storage were approximately one month "younger" or more fresh, compared to seedlings in cold storage.

For the seedlot of average quality, the treatments did not result in differences in seedling survival after outplanting at the nursery. For the five seedlots of more questionable quality, frozen storage resulted in significantly ($P < 0.01$) more seedlings rated in good or fair condition than in marginal condition or dead after outplanting.

INTRODUCTION

During the past several years at J. Herbert Stone Nursery, USDA Forest Service, in southwestern Oregon, 1+1 Douglas-fir transplants have periodically deteriorated in cold storage (33-35°F / 0.6-2°C). Pythium sp. has been associated with discoloration and death of stem and root tissues during cold storage. Unthrifty seedlings, especially with tissue damage due to mechanical or biological agents, tend to deteriorate in storage faster than thrifty seedlings. Frozen storage is a possible solution to this recurring problem.

Studies show that frozen storage (28°F / -2°C) can hold conifer seedlings in dormant, viable condition as well or better than traditional cold storage (McDonald et al., 1983; Hee 1986). In addition to saprophytic molds, several pathogens, notably species of Pythium, Cylindrocarpon, and Botrytis, can grow slowly in cold storage, causing death of tissues, especially if temperatures fluctuate above 35°F (2°C) (Hamm et al., 1990; D.Hildebrand, unpublished). Growth of these fungi is suspended below 32°F (0°C).

As of 1983, fall lifting and over-winter frozen storage was a standard practice for some stock at Coeur d'Alene Nursery, USDA Forest Service, in northern Idaho (McDonald et al., 1983). The current practice is to lift seedlings no earlier than November; pack them in plastic-lined bags; place bagged seedlings in boxes; and store them at 28°F. The boxes of bagged seedlings are shipped frozen in trucks kept at 28°F. Recipients usually hold the seedlings in

frozen storage, and thaw in cold storage prior to planting. Variable durations of cold storage of previously frozen stock is maintaining seedling quality for Coeur d'Alene Nursery and customers in the Northern Region (personal communication, Steve Omi, 1993).

Since 1978, frozen storage of conifer seedlings has been a routine practice at Weyerhaeuser forest nurseries in Washington and Oregon (Hee 1986). Hee (1986) notes that a moisture barrier is a necessary part of the packing materials. They thaw frozen stock at 10 to 15°C (50 to 59°F) on widely spaced pallets, to allow sufficient air circulation, in a warehouse or similar structure. Bareroot seedlings thaw in 3 to 5 days, while container seedlings require 10 to 15 days. Then seedlings are shipped to the customer who must plant them very soon after delivery. Quick-thawing (at temperatures above 50°F) promotes rapid development of Botrytis grey mold that seriously reduces seedling viability, especially if seedlings are not planted soon. To avoid deterioration, stock must be planted within ten days of quick-thawing (personal communication, Weyerhaeuser nursery managers).

Based on the experience at other nurseries, the expectation is that frozen storage with slow thawing in cold storage would prolong seedling viability better than cold storage for the entire time. J. Herbert Stone Nursery personnel would like to recommend frozen storage, especially for transplants. However, no data are available to predict favorable results at J. Herbert Stone Nursery. Some customers are reluctant to agree to changing to frozen storage without site-specific data.

Cold hardiness develops in response to increasing exposure to cold nights and decreasing photoperiod. The relatively mild weather at J. Herbert Stone Nursery enables the nursery to lift and store conifer seedlings when they reach deepest dormancy, in midwinter. An administrative study at J. Herbert Stone Nursery determined the optimum lifting window as mid-December to mid-February, for cold storage and spring planting (Steinfeld and Jenkinson, 1989). Seedlings lifted during this window are fully dormant and cold hardy, and are expected to be physiologically ready for frozen storage.

A potential complication with frozen storage concerns the variable weather conditions at outplanting sites. Customers sometimes want their stock as early as March or as late as May. Because favorable planting conditions often revert to inaccessible or frozen ground conditions, transplants are often stored for several weeks on the Forests or Districts. Therefore, previously frozen seedlings would be subjected to cold storage for varying lengths of time. Also, the effect of freezer breakdown, where seedlings thaw and then refreeze, on seedling viability is not known.

OBJECTIVES

The objectives of this evaluation were to address the following questions:

1. How does frozen storage compared with cold storage affect outplanting survival of 1+1 Douglas-fir transplants?
2. How does duration of cold storage affect the viability of previously frozen transplants?
3. How does re-freezing affect the viability of transplants previously frozen and thawed at 33-35°F?

METHODS

This study was initiated for 1+1 Douglas-fir transplants lifted in January 1994 at J. Herbert Stone Nursery.

Treatments

For seedlot 812 of 1+1 Douglas-fir, with average seedling quality, five treatments (illustrated on following page) included:

- 1) Control: Constant cold storage (33-35°F) for 14.5 weeks.
- 2) Frozen storage (27.5-29.5°F) for 6.5 weeks, then cold storage for 8 weeks.
- 3) Frozen storage for 6.5 weeks, then cold storage for 4 weeks, then frozen storage for 4 weeks.
- 4) Frozen storage for 10.5 weeks, then cold storage for 4 weeks.
- 5) Continuous frozen storage for 14.5 weeks.

Seedlings were lifted and packed January 13 and put in cold storage. Frozen storage began January 28, for treatments 2,3,4 and 5. Stock remained frozen until moved to cold storage on the appropriate date. Treatments ceased on May 10 when all stock was moved to cold storage, and allowed to thaw slowly. Seedlings were outplanted in the nursery in a randomized block design on May 20.

Each treatment was replicated 5 times, with 250 seedlings in a packing bag for each replicate. A sixth bag of seedlings for each treatment was used for sampling during treatment. The total number of seedlings in the study was 7500, in 30 bags.

The six bags for each treatment were kept on one rack per treatment in the storage facility. Racks were stacked together to minimize variability due to position in the storage room, and with adequate access to both sides of each rack. When seedlings needed to be moved for treatment, the entire rack was moved by forklift.

Additional Seedlots

In order to ensure that any differences between cold and frozen storage would be evident, five additional lots of 1+1 Douglas-fir transplants (811, 813, 814, 816, 823), exhibiting worse than average chlorosis and needle and twig mortality, were compared under continuous cold or continuous frozen storage. From each of the five questionable lots, two bags went into continuous frozen storage along with Treatment 5, and two bags went into continuous cold storage along with Treatment 1. The 10 bags per treatment occupied two racks. One bag supplied seedlings for examination during storage, and the other bag supplied seedlings for outplanting.

Physiological Tests

The nursery had RGP tested on December 22 (before lifting) and January 13 (at the time of lifting), on a standard sample of 80 seedlings representing the lot 812. Two samples (of 80 seedlings) were randomly collected during the packing operation according to standard nursery practice for RGP sampling. One sample was submitted immediately for RGP testing. The second sample was put in a bag

in cold storage with the rest of the seedlings. This second sample bag was moved to frozen storage on January 28, placed with treatment 3, and moved in and out of cold storage with treatment 3. On April 12, after 6.5 weeks of frozen storage and 4 weeks of cold storage, seedlings from this bag were submitted for frost hardiness testing at a commercial facility.

On May 19, samples of seedlings were taken from one replicate of treatments 1, 2, 3, and 5 for frost hardiness testing. One sample of five seedlings from each treatment was tested at each of three temperatures: 15, 20, and 25°F. Seedlings were potted and placed in a freezer at the appropriate temperature for two hours. Seedlings were then placed under growing conditions and monitored for two weeks, until June 7, 1994.

Pathological Examination During Storage

For pathological examination, the bark of the roots and shoots was scraped off with a dull scalpel to reveal the cambial layer ("scrape test"). Healthy cambium appears white, while unhealthy cambium appears orange or brown. Three parameters indicated pathological condition during storage: average percentage of necrotic roots, average number of seedlings with small patches or spots of discolored cambium (mottling) in the stem or root, and average number of seedlings with some evidence of disease in the shoot. Data was transformed as necessary to fit the assumptions of analysis of variance (anova), and each parameter was tested by two-way anova with treatment and time as the main effects.

Portions of roots and stems with abrupt transitions between healthy and diseased tissue were excised, sterilized in 10 percent bleach for three minutes, rinsed in sterile distilled water, and placed aseptically on culture medium. Culture media included 2 percent water agar, Komada's (1975) specialized medium for *Fusarium* spp, and corn meal agar modified with 20 ppm pimarin and 20 ppm vancomycin for growth of Pythiaceae fungi.

Roots of 100 seedlings, randomly collected from the sorting table to represent the entire 812 lot, were examined for pathological discoloration on January 13. One week later, a sample of 20 seedlings from each of the five questionable lots was collected from both the continuous cold and continuous frozen storage treatments, and examined.

Because opening bags during storage can be deleterious and is not an operational practice, one bag for each treatment for each of the six seedlots was designated for sampling purposes. Roots of 20 seedlings for each treatment for each seedlot were examined at four additional times: February 14, March 15, April 12, May 12. After removal of samples at each sampling time, the sample bags were resealed and returned to the racks.

This sampling scheme assumed that the greatest variation was between seedlings, and that conditions in the different bags did not vary significantly. Seedlings in bags sampled for pathological examination were expected to be in no better condition than seedlings in undisturbed bags. The pathological examinations were intended to indicate when during storage root necrosis became apparent, how fast it progressed, and whether the treatments affected disease development.

Temperature Monitoring

A thermal data probe was placed in the middle of the bag reserved for sampling for treatments 1, 2, and 3 on January 14. When samples were taken March 15, the data were downloaded to recover the temperature records. The probes were then placed in one of the undisturbed bags for each of the three treatments, because the designated sampling bags were almost half empty by then. Data from the probes were downloaded again in April and probes returned to the same bags until May 20, when they were removed and the data downloaded for the last time.

Survival after Outplanting

Each of the five undisturbed bags of seedlings for the five treatments for lot 812, was considered a replication, and 28 seedlings randomly chosen from each bag were planted as a plot. Plots were laid out in a 5x5 randomized block design, five blocks with five plots, each plot with seedlings from one bag (replication) from each of the treatments 1-5.

Seedlings from the undisturbed bag from each of the two treatments for each of the five questionable lots was also used for outplanting at the nursery. For each treatment for each lot, three replicates of 28 seedlings were planted randomly along with the treatments for lot 812. Thus each block in the field contained 11 plots, five from lot 812 and six from the additional five seedlots. The actual order of the blocks along the bed, and the plots within the blocks were assigned randomly.

On May 20, seedlings from each bag were placed in one tub, labelled, watered, covered with burlap, and tubs loaded onto pallets for planting. Seedlings were auger-planted in nursery beds, at 1 per sq ft, or 4 seedlings per linear bed-foot. Each plot occupied approximately 7 ft of bed, and each block occupied 77 bed-ft. The planting was accomplished in one day. Seedlings were watered at the time of planting, and not watered again until pre-dawn PMS (plant moisture stress) readings were at 15 to 20 bars. Plots were weeded as necessary, and no fertilizer, root pruning, or other cultural practices were employed.

Beginning two weeks after planting, seedlings were monitored approximately every four weeks through August, for budbreak (separation of bud scales revealing emerging needles), and mortality.

At the final scoring in October 1994, seedlings were rated as

Good = Set bud, foliage mostly green

OK = Set bud, some branch mortality, "ugly, but will survive"

Marginal = Extensive branch mortality, few live buds, "might survive"

Dead = Completely red or withered foliage, or no live buds

Survival of lot 812 was tested by two-way anova with the five treatments and the five blocks as the main effects. For the five additional seedlots with just cold and frozen treatments, three plots of treatment 1 (continuous cold storage) and 5 (continuous frozen storage) from seedlot 812 were chosen randomly and added to the data set as a sixth seedlot. Survival of the six seedlots was tested by two-way anova with treatments (cold or frozen) and seedlot as the main effects, with 3 samples.

RESULTS

Physiology

For lot 812, RGP was reported as "excellent" two weeks before lifting (December 22, 1993) and at lifting (January 12, 1994). Seedlings from Treatment 3 (frozen storage for 6.5 weeks, cold storage for four weeks--until April 12, then frozen storage for four weeks) were frost hardy to -12.5°C on April 13. The day before outplanting, seedlings from treatments 1,2,3, and 5 were tested for frost hardiness. At the lowest temperature tested, -9.4°C (15°F), no mortality was observed, but 34 to 94 percent of the foliage was killed (Table 1).

Table 1. Results from frost hardiness testing begun May 19, 1994, for seedlot 812 (of average seedling quality) after five storage treatments: Average percent of seedlings with dead terminal buds, average percent of dead stems and dead foliage, average percent of seedlings with brown foliage tips, with budbreak, and with root growth, two weeks after exposure to three temperatures, averaged for five seedlings per temperature.

Test $^{\circ}\text{C}$	Temp. $^{\circ}\text{F}$	Dead Terminal Buds	Dead Stem	Dead Foliage	Brown Tips	Budbreak	Root Growth
Treatment 1 (continuous cold storage)							
-3.9	25	0	0	0	20	20	100
-6.7	20	0	0	21	0	80	0
-9.4	15	0	3	94	0	100	0
Treatment 2 (six weeks frozen storage, then 8 weeks cold storage)							
-3.9	25	0	0	0	0	0	60
-6.7	20	0	0	0	60	100	100
-9.4	15	20	0	34	0	100	40
Treatment 3 (six weeks frozen, 4 weeks cold, then 4 weeks frozen storage)							
-3.9	25	0	0	0	0	0	80
-6.7	20	0	0	6	40	60	80
-9.4	15	0	0	55	0	100	40
Treatment 5 (continuous frozen storage)							
-3.9	25	0	0	0	0	40	60
-6.7	20	0	0	3	60	100	80
-9.4	15	0	0	75	0	100	0

Temperature

Within-bag temperature in Treatment 1 reached 1°C (34°F) 16 hours after the thermal data probe was activated. From January 14 through May 20, temperatures recorded by the probe ranged from 27 to 34°F (-2.5 to 1°C). Average within-bag temperature for Treatment 1 as recorded by the probe was approximately 31°F (0.5°C). Temperatures recorded by a digital thermometer mounted on the back wall of the storage unit were read once per day. The average storage temperature as reported by the wall thermometer was 32.3°F, with a range of 30 to 35°F. Given the variation between thermometers, the actual temperatures probably averaged a little colder than the temperature range usually reported for cold storage--33 to 35°F. Ice was not observed in or around the seedling bags at sampling times during cold storage.

Within-bag temperature in Treatment 2 reached 30°F (-1°C) on January 31, three days after freezing began. During frozen storage, within-bag temperatures averaged 28°F (-2.2°C). Cold storage began March 15, and within-bag temperatures rose above freezing eight days later, on March 23 (32.9°F/0.5°C). During cold storage, within-bag temperatures averaged 34 to 35°F (near 1.5°C).

Within-bag temperature in Treatment 3 reached 30°F (-1°C) on January 28, approximately 16 hours after freezing began. During frozen storage until March 15, within-bag temperature averaged approximately 27.5°F (-2.5°C). After cold storage began on March 15, within-bag temperature rose above freezing ten days later on March 25, and then averaged 33 to 34°F (1°C) for 18 days until April 12. Freezing resumed on April 12, and within-bag temperature reached 30°F after 2 days. During frozen storage until May 10, within-bag temperatures averaged approximately 29°F (-1.5°C).

Bags of seedlings in the frozen storage treatment were kept deep inside a large commercial freezer. Bags for Treatment 4 (frozen storage until April 12, then cold storage) were moved to a smaller commercial refrigerator and placed fairly close to the door. When the door was open, large vertical plastic panels helped keep the cold air inside. In mid to late April there was much fork lift activity and the door was often open. At the time of the April sampling, sunlight was shining on the treatment bags through the open door. Whether the temperature within the bags rose above 35°F is not known.

Pathology

At the beginning of storage in January 1994, the percentage of roots diseased averaged five percent or less for all six seedlots. For seedlot 812 (of average quality) the five cold and frozen storage treatments did not result in differences in percentage of roots diseased, or of percentage of seedlings with spots or small patches of necrotic cambium (mottling) by May 1994.

For the other five seedlots (of questionable viability), the percentage of roots diseased tended to increase with time in cold storage while remaining constant in frozen storage. Table 2 includes results from seedlot 812, treatments 1 (continuous cold storage) and treatment 5 (continuous frozen storage) along with the other five seedlots. Statistical comparisons were not made because data transformations could not bring the data to normal distribution with homogeneous variance.

For all seedlots, the percentage of seedlings with mottling was significantly greater ($P < 0.01$) after three months of cold storage, compared with that after three months of frozen storage. In April, an average of 71% of seedlings had mottling after cold storage, while after frozen storage, an average of 32.5% of seedlings had mottling (Table 2). By May, the proportion of seedlings with mottling had increased in frozen storage, but was still lower than the proportion of mottled seedlings in cold storage in April. With regard to mottling, seedlings in frozen storage were approximately one month "younger" or more fresh, compared to seedlings in cold storage. Mottling was considered an indicator of seedling vigor, with more mottling meaning increased tissue necrosis and increased susceptibility to pathogen invasion.

Occasionally, wefts of mycelium were apparent scattered on a seedlings roots. Saprophytic molds and pathogens are always present, at least as a thin film, in soil adhering to seedling surfaces. Some of these fungi can produce visible mycelium during cold storage. Beneficial mycorrhizal fungi can also produce wefts of mycelium during storage. The presence of visible mycelium on seedlings is not a reliable indicator of seedling tissue condition.

There were no differences between treatments in the numbers of seedlings with necrosis or other problems in the shoot.

Isolations from sloughing and/or discolored fine roots in January yielded species of Pythium, Fusarium, and Phytophthora. In March and April, isolations from sloughing and/or discolored stem and root tissue consistently yielded species of Pythium and usually also Fusarium. Species of Cylindrocarpon and Botrytis were not detected.

Survival

Mortality of outplanted seedlings due to dessiccation seemed to occur steadily as the summer progressed. Many seedlings flushed and then died back.

For seedlot 812, of average quality, the five cold and frozen storage treatments did not result in differences in percent of seedlings that were rated in good, fair, or marginal condition, or were dead at the end of summer after outplanting at the nursery (Table 3).

Analysis of the five seedlots of more questionable quality showed a significant treatment effect. Frozen storage resulted in significantly ($P < 0.01$) more seedlings rated in good or fair condition than in marginal condition or dead (Table 4).

Table 2. Average percentage of root system with root disease (%RD), percentage of seedlings with mottling (Mottled), and overall average percentage of root disease (Average %RD) and percentage of seedlings mottled (Average Mottled) for 1+1 Douglas-fir transplants under cold and frozen storage treatments, in March, April, and May 1994.

CONSTANT COLD			CONSTANT FROZEN	
Seedlot	%RD	Mottled	%RD	Mottled
<u>MARCH</u> (after 6.5 weeks)				
811	<5	60	<5	15
812	<5	30	<5	40
813	<5	20	<5	20
814	<5	35	<5	30
816	<5	30	<5	40
823	5.6	20	<5	20
Overall				
Average	<5	31.5	<5	26.5
<u>APRIL</u> (after 10.5 weeks)				
811	<5	60	<5	15
812	<5	85	<5	20
813	14.9	85	<5	15
814	<5	80	<5	55
816	5.3	65	<5	45
823	<5	50	<5	45
Overall				
Average	5.8	71 *	<5	32.5*
<u>MAY</u> (after 14.5 weeks)				
811	<5	70	<5	55
812	<5	60	<5	55
813	19.8	85	<5	50
814	9.1	70	<5	80
816	8.9	80	<5	75
823	6.8	80	<5	60
Overall				
Average	8.5	74	<5	62.5

*Significantly different at $P < 0.01$

Table 3. Average percentage of seedlings from seedlot 812 rated in good, fair, or marginal condition, or dead in October after outplanting at J. Herbert Stone Nursery, compared between five storage treatments.

Storage Treatment	Good	Fair	(Good + Fair) Satisfactory	Marginal	Dead	(Marginal + Dead) Unsatisfactory
1. Continuous cold storage	55.7	9.3	65.0	3.6	31.4	35
2. Frozen for 6.5 weeks, then cold 8 weeks.	75.0	3.6	78.6	0	21.4	21.4
3. Frozen 6.5 weeks, cold 4 weeks, frozen 4 weeks.	71.4	4.3	75.7	2.1	22.1	24.3
4. Frozen 10.5 weeks, then cold 4 weeks.	55.7	9.3	65.0	0.7	34.3	35.0
5. Continuous frozen storage.	70.7	7.9	78.6	1.4	20.0	21.4

Table 4. Average percentage of seedlings from six seedlots rated in good, fair, or marginal condition, or dead, in October, five months after outplanting at J. Herbert Stone Nursery, compared after 14.5 weeks of cold or frozen storage prior to outplanting.

Seedlot	Storage Treatment	Good	Fair	(Good + Fair) Satisfactory	Marginal	Dead	(Marginal + Dead) Unsatisfactory
811	Cold	51.1	6.0	57.1	3.6	39.3	42.9
811	Frozen	61.8	1.1	63.2	2.5	34.6	36.8
812	Cold	57.1	6.1	63.2	5.7	31.1	36.8
812	Frozen	63.2	10.7	73.9	2.5	23.6	26.1
813	Cold	35.7	2.5	38.2	3.6	58.2	61.8
813	Frozen	82.1	1.1	83.2	2.5	14.3	16.8
814	Cold	40.4	3.5	43.9	2.5	53.6	56.1
814	Frozen	53.6	1.0	54.6	3.6	41.8	45.4
816	Cold	28.6	4.6	33.2	0.0	66.8	66.8
816	Frozen	61.8	6.1	67.9	0.0	32.1	32.1
823	Cold	22.5	14.3	36.8	0.0	63.2	63.2
823	Frozen	56.1	5.7	61.8	2.5	35.7	38.2
Overall	Cold	39.2	6.2	45.4*	2.6	52.0	54.6*
Average	Frozen	63.1	4.3	67.4*	2.3	30.3	32.6*

*Significant differences at $P < 0.01$.

DISCUSSION AND SUMMARY

The average temperature during the constant cold storage, 31 to 32.3°F, was somewhat colder than usual, which decreased the differences between treatments. Cold storage temperatures closer to 35°F might have resulted in greater differences in seedling response between treatments.

Root discoloration and necrosis tended to increase more under cold storage than frozen storage, and this effect was more evident in seedlots of below average quality. Tissue discoloration is an indicator of susceptibility to pathogen invasion.

This study showed no significant differences between various durations of cold storage after freezing, including thawing and refreezing, for a seedlot of average quality.

Although outplanting at the nursery is not equivalent to outplanting in the forest, seedlings were subjected to sufficient heat and moisture stress to bring out differences in seedling viability due to treatment effects. The results of this study indicate that seedling survival can be improved by frozen storage compared to cold storage, and that the difference in survival is more pronounced for stock that show worse than average chlorosis and needle and twig mortality.

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